Constitutive activity of the human histamine H_4 receptor: Computational studies on wild-type and mutant H_4R orthologs

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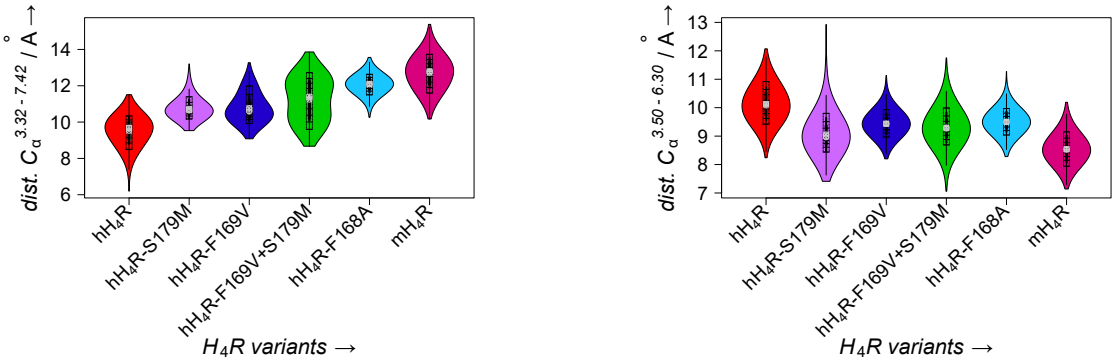
Histamine H_4 receptor (H_4R) orthologs are G-protein coupled receptors (GPCRs) that exhibit species-dependent constitutive (basal) activity: In contrast to mouse H_4R (mH_4R), human H_4R (hH_4R) shows a high degree of constitutive activity.

In a previous molecular-pharmacological study, we characterized the constitutive activity of hH_4R, mH_4R as well as a series of hH_4R mutants, comprising hH_4R-S179M, hH_4R-F169V, hH_4R-F169V+S179M [1] and hH_4R-F168A [2]. An exchange of $F169^{ECL2}$ to V significantly decreased the constitutive activity compared to wild-type hH_4R, while that of the hH_4R-S179M mutant is similar to that of hH_4R. [1] Remarkably, the basal activity of the hH_4R-F169V+S179M [1] and hH_4R-F168A [2] mutants is even comparable to that of mH_4R.

Hence, though we identified residues that account for the high constitutive activity of the hH_4R, the underlying molecular mechanism by which the basal equilibrium between inactive and active receptor states is shifted towards the inactive state is still unknown. To shed light on this matter, we have performed long-time-scale (2 µs) molecular-dynamics simulations on wild-type hH_4R, the hH_4R mutants S179M, F169V, F169V+S179M, F168A, and on mH_4R.

During the MD simulations, $F169^{ECL2}$ is dipping into the binding pocket merely in case of hH_4R and is thereby interacting with the surrounding aromatic and hydrophobic residues. Interestingly, F169 seems to take the role of an agonist, thus contributing to the stabilization of the active state. As a measure of binding pocket contraction, the distance (C_α) between $D94^{3.32}$ and $Q347^{7.42}$, starting at approximately 11 Å, increased by a maximum of ~3 Å for the hH_4R mutants and mH_4R, while, by contrast, it decreased by up to 3 Å for the basally active hH_4R. At the intracellular side, initial C_α-C_α distances of around 8.0 Å between $R112^{3.50}$ and $A298^{6.30}$ increased more for hH_4R than for the hH_4R mutants and mH_4R, thus showing an enhanced outward movement of TM6 for hH_4R compared to the other H_4R variants. This is in accordance with the fact that GPCR activation is reflected by a subtle contraction of the orthosteric binding pocket and a notable outward motion of TM6 at the intracellular side.



Hence, H_4R variant-dependent differences between essential motifs of GPCR activation correlate with experimentally determined constitutive activities and provide a molecular explanation for the differences in constitutive activation. Furthermore, the results shed new light on the molecular mechanism of basal H_4R activation that are of importance for other GPCRs.

[1] D. Wifling, et al., *Br. J. Pharmacol.*, **2015**, 172, 785-798.
[2] D. Wifling, et al., *PLoS One*, **2015**, 10, e0117185.